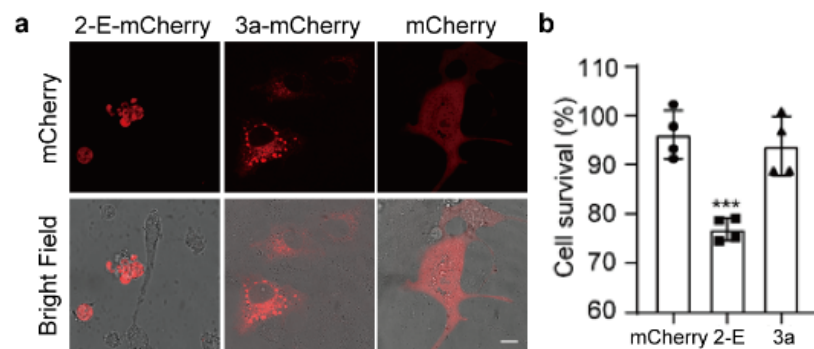


# 1 **Supplementary Figures and Videos:**

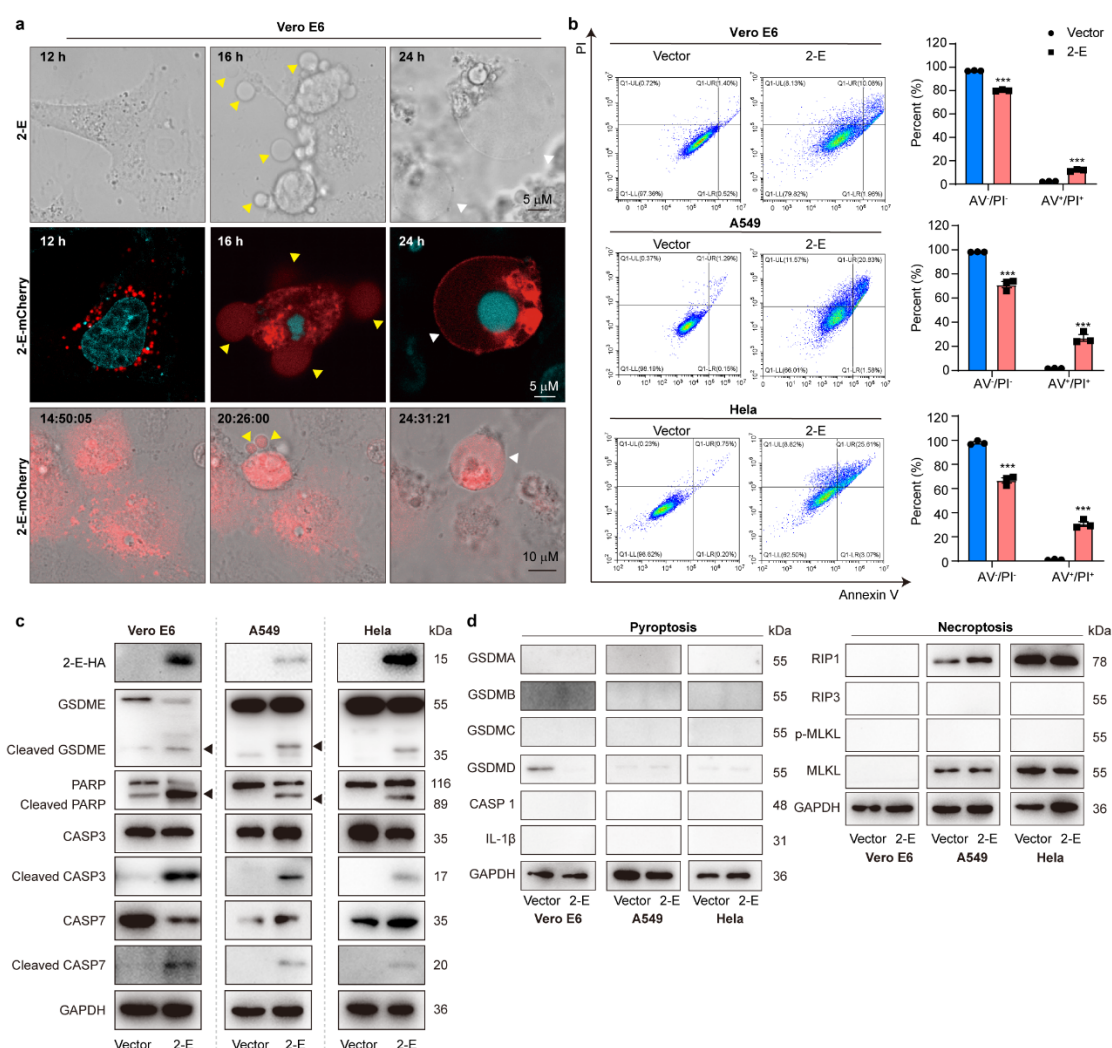


2

3 **Supplementary Fig. S1: Cell images and viability level after transfection as**  
 4 **indicated.**

5 **a**, Images of Vero E6 after transfected with 2-E channel, 3a channel and mCherry (bar,  
 6 10  $\mu$ m). The controls 3a and mCherry did not induce blebbing and cell death. **b**, Cell  
 7 survival for Vero E6 cells after transfected with 2-E channel, 3a channel and mCherry  
 8 for 24 h. Shown were the mean  $\pm$  SEM of three independent experiments.  $*p < 0.05$ ,  $*$   
 9  $*p < 0.01$ ,  $***p < 0.001$ ; unpaired Student's t test.

10

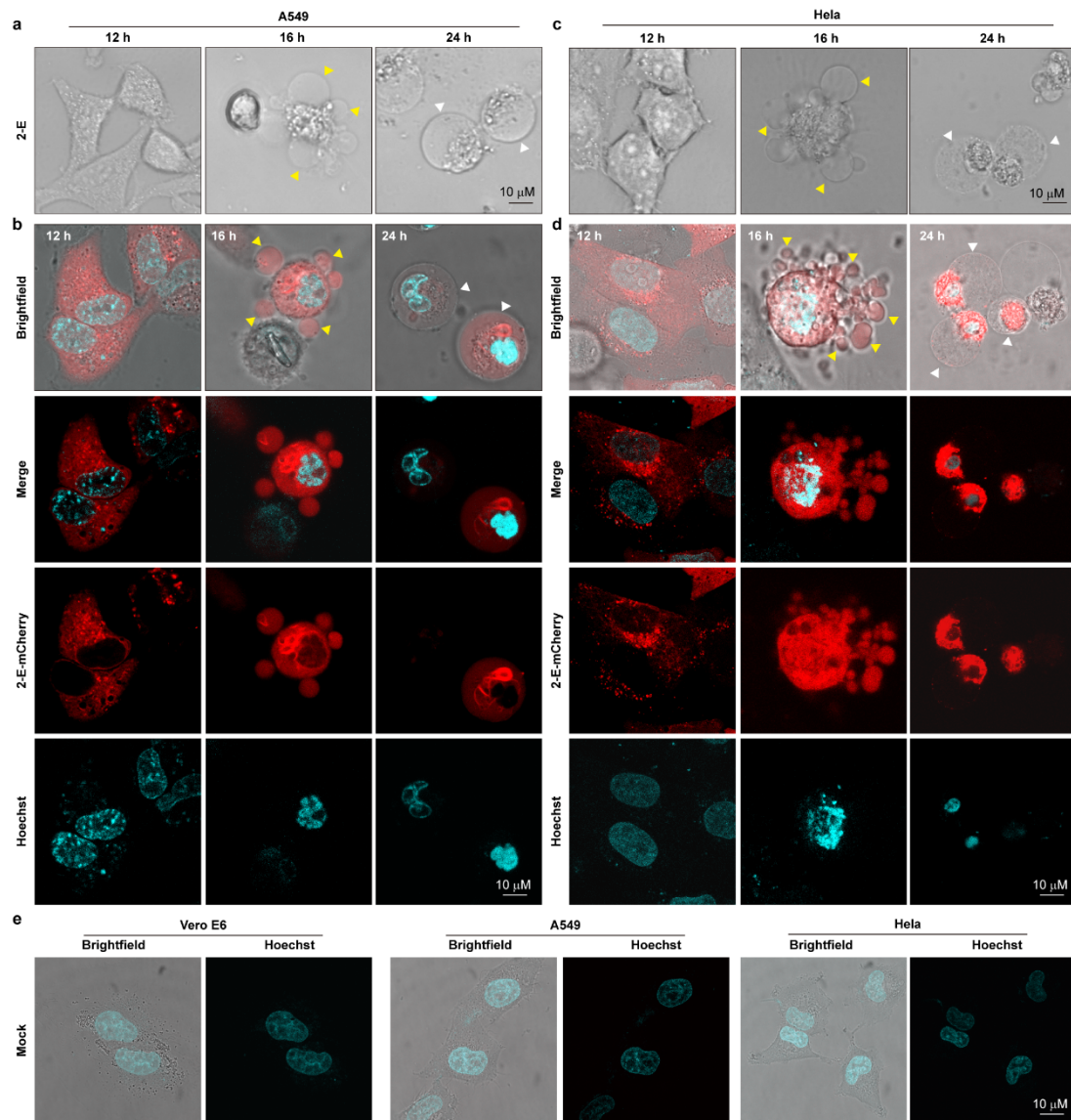


**Supplementary Fig. S2: 2-E induces pyroptosis via GSDME cleavage.**

**a**, Images of 2-E expressing Vero E6 cells at different transfection point (up). Fluorescent images of Vero E6 cells after transfection with 2-E-mCherry and stained with Hoechst (middle). Representative time-lapse cell images (fluorescence and bright field) were taken from 12-24 h after 2-E-mCherry transfection (bottom). Arrowheads indicate ballooned pyroptotic cells and secreted vesicles. **b**, Three cell lines transfected with 2-E plasmids respectively. Flow cytometry analysis of Propidium iodide (PI) and Annexin V (AV) stained. **c**, Immunoblotting of 2-E-induced GSDME cleavage by caspase-3 and caspase-7 in three types of cells. **d**, Immunoblotting of cell death pathway

21 biomarkers in 2-E transfected cells. Shown were the mean  $\pm$  SEM of three independent  
22 experiments.  $^*p < 0.05$ ,  $^{**}p < 0.01$ ,  $^{***}p < 0.001$ ; unpaired Student's t test.

23



24

25 **Supplementary Fig. S3: Images of 2-E expression HeLa and A549 cell respectively.**

26 **a**, Images of 2-E expressing A549 cells at different transfection point. **b**, Representative

27 A549 cell images (fluorescence and bright field) were taken from 12-24 h after 2-E-

28 mCherry transfection. Hoechst staining (Blue) indicates nucleus. Arrowheads indicate

29 ballooned cell membrane characteristic of pyroptotic cells. **c**, Images of 2-E expressing

30 HeLa cells at different transfection point. **d**, Representative HeLa cell images

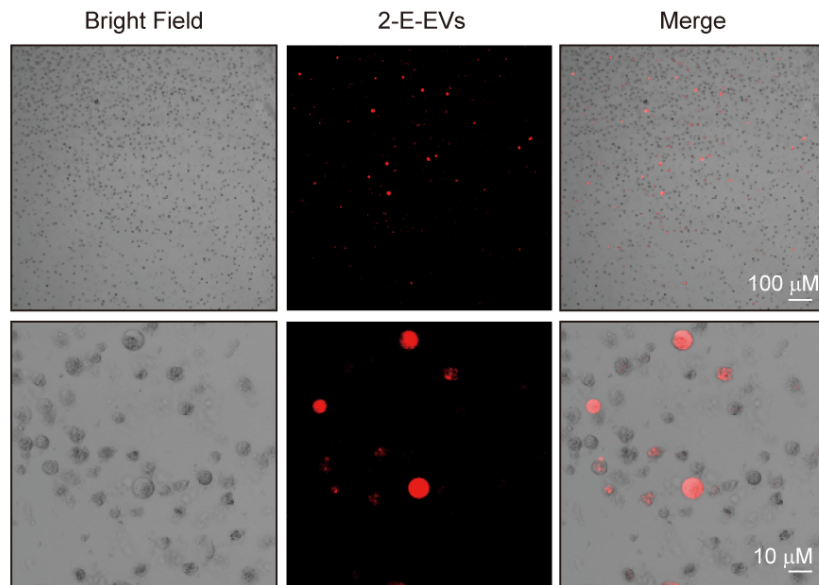
31 (fluorescence and bright field) were taken from 12-24 h after 2-E-mCherry transfection.

32 Hoechst staining (Blue) indicates nucleus. Arrowheads indicate ballooned cell

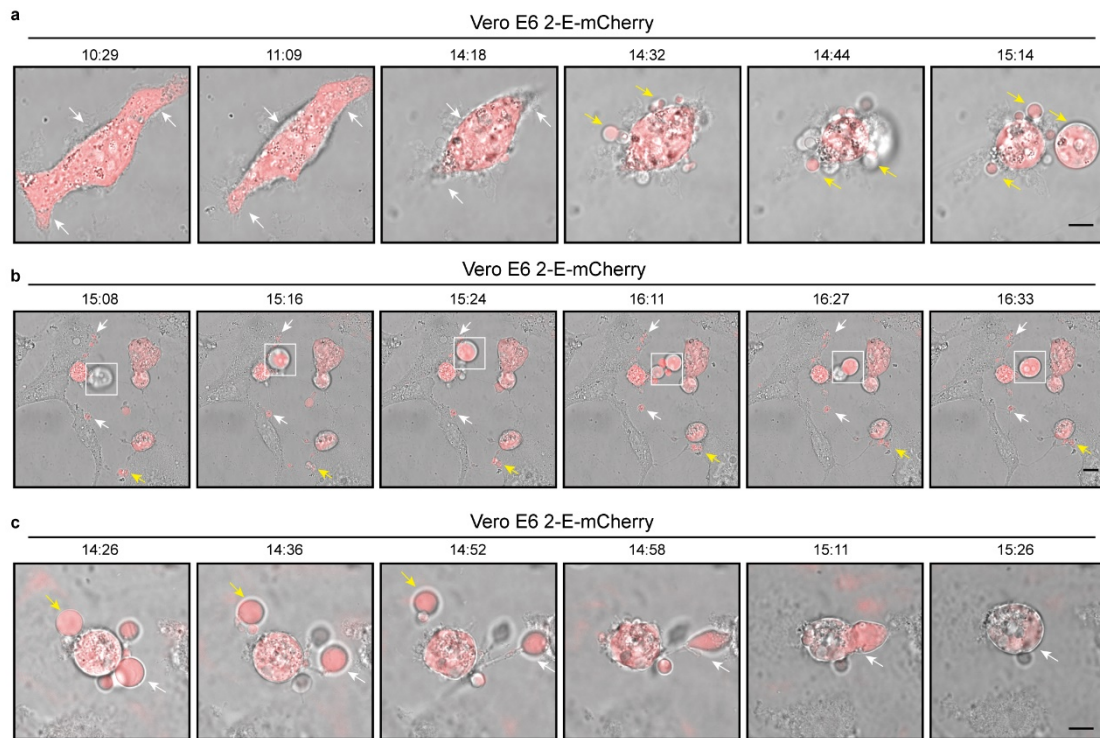
33 membrane characteristic of pyroptotic cells (a-d). **e**, Images of Vero E6, A549 and Hela

34 cells transfected with vectors as control.

35



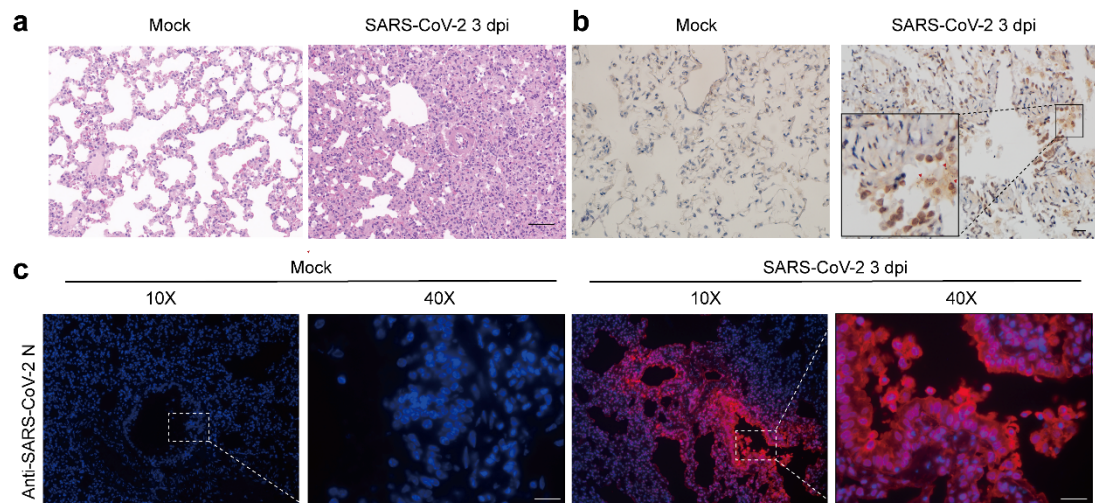
**Supplementary Fig. S4: Microscopy images of 2-E-induced EVs.** Vero E6 cells transfected with 2-E-mCherry plasmid and 2-E-EVs were isolated from the transfected cells.



**Supplementary Fig. S5: Microscope images of Vero E6 cells transfected with 2-E-mCherry.**

**a-c**, Representative time-lapse cell images (fluorescence and bright field) were taken from 10-16 h after transfection (bar, 10  $\mu$ m). **a**, White arrow, healthy cell became swelling. Yellow arrow, multiple EVs secreted. **b**, White arrow, attached EVs. Rectangle, fused EVs. Yellow arrows, rope network. **c**, Yellow arrow, the EVs shed from their original cell and floated away. White arrow, the EVs were pulled backed by the elastic rope and fused with its original cell.

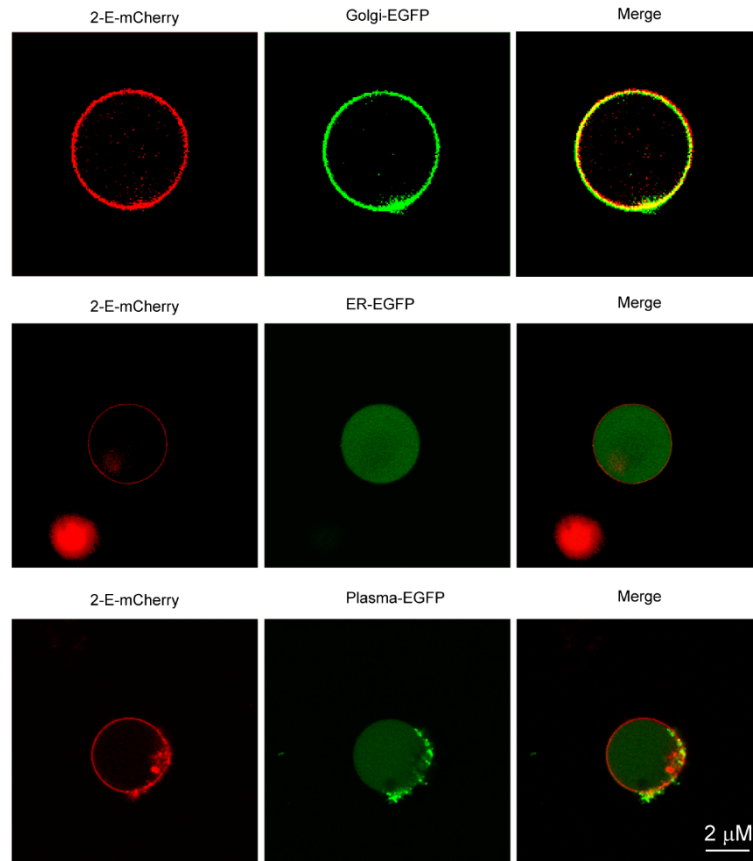




**Supplementary Fig. S6: Histopathology of lungs in golden hamster infection models.**

**a**, HE staining images of golden hamster lung tissues infected with SARS-CoV-2 or not. (bar, 20  $\mu$ m) **b**, Immunohistochemistry analysis of SARS-CoV-2 N protein in SARS-CoV-2 infected and healthy golden hamster lung tissues (bar, 20  $\mu$ m). **c**, Immunofluorescent analyzed of SARS-CoV-2. Blue, DAPI; Red, SARS-CoV-2 N protein, which indicated viral infected cells (bar, 20  $\mu$ m).

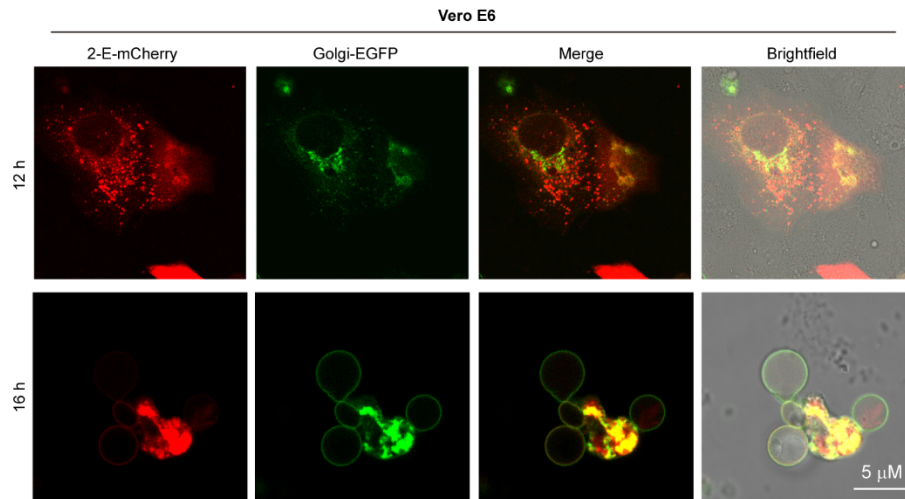




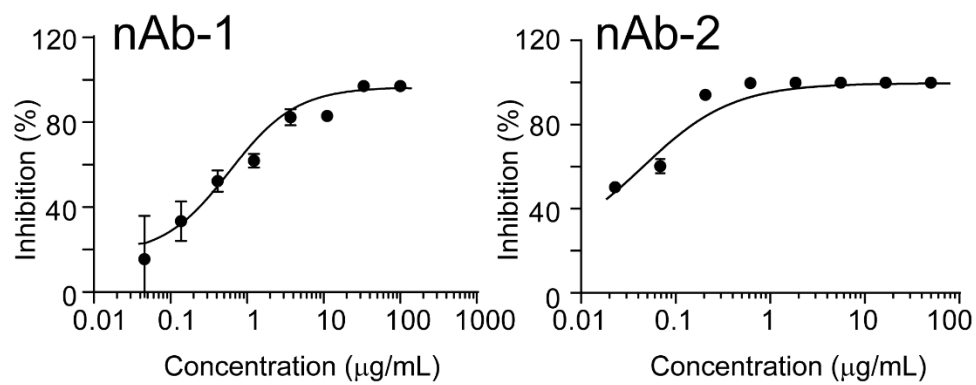
60

61 **Supplementary Fig. S7: Images of Vero E6 cells after co-transfected 2-E and Golgi-**  
 62 **EGFP, ER-EGFP or Plasma-EGFP markers. 2-E-EVs were isolated from the**  
 63 **transfected cells.**

64



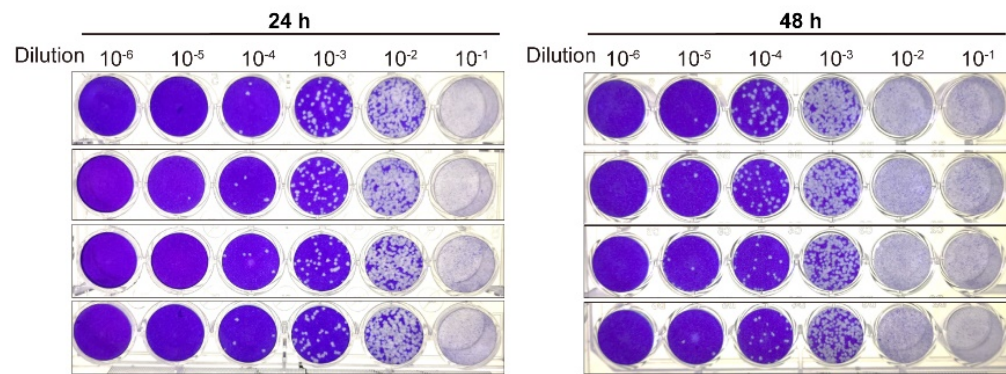
**Supplementary Fig. S8: 2-E localizes on Golgi apparatus and forms EVs.** Vero E6 cells co-transfected with 2-E-mCherry and Golgi-EGFP (B4GALT1-EGFP, the marker of Golgi). The images were captured at indicated time after transfection.



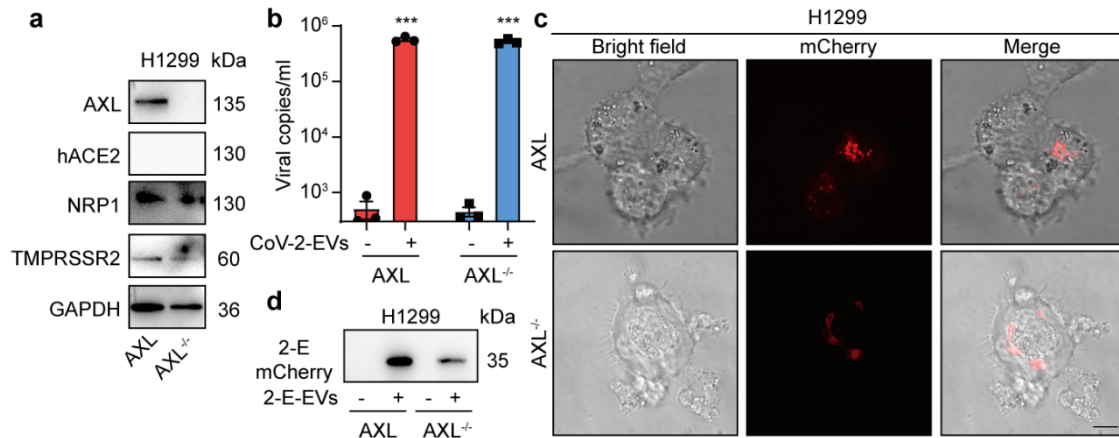
70

71 **Supplementary Fig. S9: IC<sub>50</sub>S of two SARS-CoV-2 neutralizing antibodies under**  
 72 **0.01 MOI live virus infection.**

73



**Supplementary Fig. S10: Plaque reduction assay of SARS-CoV-2 induced EVs re-infecting Vero E6 cells for 24 and 48 h.**

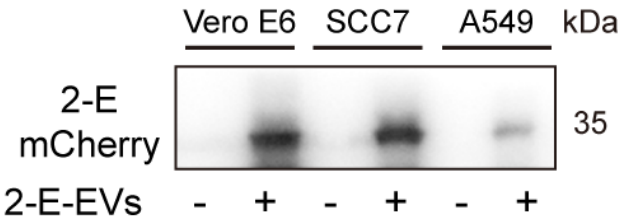


**Supplementary Fig. S11: SARS-CoV-2-induced EVs infected H1299 cells independent of AXL receptor.**

**a**, Expression of AXL, hACE-2, NRP-1 and TMPRSSR2 in H1299 and H1299 AXL<sup>-/-</sup> cells. **b**, H1299 and H1299 AXL<sup>-/-</sup> cells were incubated with isolated CoV-2-induced EVs and supernatant viral copies was quantified by qRT-PCR (n > 3 independent experiments). **c**, Images of H1299 and H1299 AXL<sup>-/-</sup> cells that were exposed to 2-E-mCherry-induced EVs (bar, 10 μm). **d**, Immunoblots detection of 2-E-mCherry in the secondary infected cells. H1299 and H1299 AXL<sup>-/-</sup> cells were exposed to 2-E-mCherry-induced EVs for 24 h. Shown were the mean ± SEM of three independent experiments.

\**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001; unpaired Student's *t* test.

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91

92 **Supplementary Fig. S12: Immunoblots detection of 2-E-mCherry in the secondary**  
93 **infected cells. Vero E6, A549 and SCC7 cells were exposed to 2-E-mCherry-**  
94 **induced EVs for 24 h.**

**Supplementary Video S1: Representative time-lapse cell images (fluorescence and bright field) were taken from 12-26 h after 2-E-mCherry transfection, related to Supplementary Fig.S2.** The morphology of the cell was observed by Leica SP8 STED confocal microscope. Images were taken every 30 s.

**Supplementary Videos S2-S3: Representative video of the Vero E6 cells blebbing process after 2-E-mCherry transfection, related to Fig.1d, Supplementary Fig.S5.**

**Supplementary Video S4: Images of Vero E6 exposed to 2-E-mCherry-induced EVs, related to Fig.5d first line.** Naïve Vero E6 cells were exposed to red EVs. The constructed 3D videos supported the presence of intact EVs in cells.

**Supplementary Video S5: Images of Vero E6 exposed to 2-E-mCherry-induced EVs, related to Fig.5d second line.** Naïve Vero E6 cells were first transfected with EGFP plasmids and then exposed to red EVs. The constructed 3D videos supported the presence of intact EVs in cells.